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CARDIOPROTECTIVE EFFECT OF GEMMOTHERAPEUTICALLY TREATED *WITHANIA SOMNIFERA* AGAINST CHEMICALLY INDUCED MYOCARDIAL INJURY

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Abstract

The present study was designed to evaluate the preventive and curative cardioprotective potential of native and gemmotherapeutically treated on the basis of biochemical, histopathological and antioxidant parameters in the salbutamol (albuterol) induced myocardial necrosis in rabbits. Gemmotherapy is a newly emerging way of treatment and no work so far has been done on evaluation of curative cardioprotective potential of *Withania somnifera.* Rabbits were divided into eight main groups: Normal, Ischemia, *WS* Gemmo-baseline, *WS* Native-baseline, *WS* Gemmo-preventive, *WS* Native-preventive, *WS* Gemmo-curative and *WS* Native-curative groups. Gemmo and native-baseline groups were treated with gemmo and native *W. somnifera* (50 mg/kg) for 20 days. Native and Gemmo-preventive groups were pre-treated with native and gemmotherapeutically treated *W. somnifera* at a dose 50 mg/kg for 3 weeks. On 20th and 21st day animals of all groups except normal and baseline groups were given *Salbutamol* (50mg/ kg), orally at an interval of 24 h. The Native-curative and Gemmo-curative groups were then treated with native and gemmotherapeutically treated *W. somnifera* at a dose 50 mg/ kg for 5 days, subsequent to the treatment with *Salbutamol*. Rabbits were subsequently sacrificed for gross pathological studies and hearts were removed for antioxidant assay. Administeration of *Salbutamol* significantly increased ($p<0.05$) the serum level of CK-MB, LDH, SGOT and SGPT in ischemia group as compared to Normal. However, pre- and post-treatment with Native and Gemmo extracts of *W. somnifera* significantly restored and reduced ($p<0.05$) the elevated serum levels of these cardiac markers. Also Native and Gemmo extracts of *W. somnifera* significantly increased ($p<0.05$) antioxidant enzymes; superoxide dismutase, catalase and glutathione peroxidase. No significant change was observed in the activity of cardiac enzymes in baseline groups. Protective actions of *W. somnifera* on heart have also been confirmed by gross pathology.

Introduction

Myocardial infarction is the leading cause of death in the world and it is the most lethal manifestation of cardiovascular diseases. It has been the object of intense investigation by clinicians and basic medical scientists (Bolli, 1994). The Indian subcontinent (including India, Pakistan, Bangladesh, Sri Lanka, and Nepal) has among the highest rates of cardiovascular disease (CVD) globally. Many reports have highlighted the high CVD rates among south Asian immigrants living in western countries, but the enormous CVD burden within the Indian subcontinent itself has been underemphasized (Goyal & Yusuf, 2006).

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Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The medicinal plants have immensely contributed to the health needs of humans throughout their existence. Even today, almost one quarter of prescribed medicines in the world control ingredients from plant origin. These are used as a major source of drugs for the treatments of various health disorders (Iqbal & Rehman, 2004). Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects. Nowadays, it is being realized that herbs can protect the heart from heart diseases by their crdioprotective action by providing an integrated structure of nutritional substances mainly phytochemicals which help in restoring and maintaining balanced body systems (Dhar *et al.,* 1968; Hertog *et al.,* 1993).

Gemmotherapy is a lesser known, modern and exciting research division of European plant or phytotherapy, which uses stem cells of buds and young shoots of trees and shrubs, gathered in the springtime when they are at a key stage of their natural growth cycle. Young shoots and buds of medicinal plants are freshly prepared in a process using water, glycerine and alcohol. Since the extracts are from fresh and growing tissues, Gemmotherapy remedies are unique in their intense combination of the vitamins, minerals and other powerful properties of the whole plant, including the flowers, leaves, fruits, sapwood and roots. They also incorporate many active principles, such as nucleic acids and growth hormones, which are no longer present when the plant fully develops. (http://www.vieharmony.com). *Withania somnifera* (WS), also known as Asgandh (Dutta, 1976), Indian gienseng and winter cherry, has been an important herb in the Ayurvedic and indigenous medical system for over 3000 years. The root of the plant is categorized as rasayanas, which is reputed to promote health and longevity by augmenting defense against disease, arresting the aging process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental well-being. (Weiner & Weiner, 1994) *W. somnifera* is a commonly used herb in Ayurvedic medicine. Studies indicate that Asgandh possesses antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinsonian, antivenom, anti inflammatory and anti-tumor properties. Various other factors like immunomodulation, hypolipidemic, antibacterial, cardiovascular protection, sexual behavior, tolerance and dependence have also been studied. (Gupta & Rana, 2007). Chemical constituents of *W. somnifera* are always of an interest for the researchers. The biologically active chemical constituents are alkaloids (ashwagndhine, cuscohygrine, ana hygrine, tropine *etc.*), steroidal compounds, including ergostan type steroidal-lactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone *etc.* (Elsakka *et al.,* 1990) As an antioxidant, WS and active constituents sitoindosides VII-X and withaferin A (WA) have been proven to increase levels of endogenous superoxide dismutase, catalase, and ascorbic acid, while decreasing lipid peroxidation (Bhattacharya *et al.,* 1997).

The present study was designed to evaluate the efficacy of preventive and curative cardioprotective potential of gemmotherapeutically treated *W. somnifera* in the experimental model of *salbutamol*-induced myonecrosis as compared to native extract of *W. somnifera*.

Materials and Methods

Plant material: Whole plant samples of *W. somnifera* were collected from the botanical garden of University of Agriculture, Faisalabad and got identified by plant taxonomist,

Department of Botany, University of Agriculture Faisalabad. Fresh growing leaves collected in spring season were used to prepare gemmo-modified plant extract, while old (native) leaves were dried and used afterward for extract preparation.

Gemmotherapy treatment of fresh plant: The method described by Churchill (2002) was used for gemmotherapeutical treatment of fresh plants. Fresh growing leaves were washed with distilled water and weighed. The sample was set aside so that water might be evaporated and the exact weight of plant material was determined. The fresh plant material was blended in a mixture of alcohol and glycerine having a ratio of 2:1 respectively. The mixture was left to stand for one month in a cool, shaded environment, and shaken from time to time to help the maceration process. It was then filtered under constant pressure. After standing for a further 48 hours, it was filtered once more. The resulting liquid was known as the stock solution, it consists of glycerine and alcohol in a ratio of 2:1 respectively. The stock solution then evaporated in rotary so that all the alcohol was removed. Then the solution was kept in an incubator at a temperature of 65° C and thus the remaining alcohol was evaporated to obtain semi-solid extract.

Water extract of native plant: 50 g of dry powdered leaves sample were boiled in 250 mL of H_2O for half an hour. This solution was left to stand for 24 h. Next day the solution was filtered and evaporated to obtain dry extract for dose preparation of rabbit.

Phytochemical analysis: Qualitative and quantitative analysis was done for identification of the major phytoconstituents of *W. somnifera* and was carried out as described by Brain & Turner (1975) and Siddiqui & Ali (1997).

Experimental animals: Twenty four rabbits weighing about 1.25 kg were used in the study. Throughout the investigation, animals were housed in a room at normal temperature with free access of food and water, and on standard conditions. Animals were weekly weighed.

Evaluation of cardioprotective activity of *W. somnifera* - Treatment protocol: The animals were randomly allocated into eight groups comprising three animals in each (Table 1).

Collection of blood samples: Blood was taken from the jugular vein of overnight fasted rabbits. The blood sample were collected in centrifuged glass tubes, then centrifuged the blood and collected the serum, separated and stored in a deep freezer for further biochemical measurements.

Biochemical analysis: Serum was used for the assay of biochemical parameters including creatine kinase (CK-MB), lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGOT) using kits and Bioanalyzer (Latest model of Smear).

Antioxidant Enzyme Analysis - Tissue homogenate preparation: A 10 % homogenate of myocardial tissue was prepared in 50mM phosphate buffer, pH 7.4. The homogenate was centrifuged at 7000 rpm for 15 min., and supernatant was used for the estimation of antioxidant enzymes (Catalase, Superoxide dismutase and Peroxidase)

Table 1. Treatment protocol.

Group	Days 1-20	Day 20th	Day 21st	5 days after ischemia
1) Normal	Normal diet	Normal diet	Normal diet	-
2) Ischemia	Normal diet	Salbutamol (50 mg/ kg)	Salbutamol (50 mg/ kg)	-
3) *WS* Gemmo baseline	Gemmo-extract of *WS* (50 mg/ kg)	-	-	-
4) *WS* native baseline	Native-extract of *WS* (50 mg/ kg)	-	-	-
5) *WS* Gemmo-preventive	Gemmo-extract of *WS* (50 mg/ kg)	Salbutamol (50 mg/ kg)	Salbutamol (50 mg/ kg)	-
6) *WS* Native-preventive	Native-extract of *WS* (50 mg/ kg)	Salbutamol (50 mg/ kg)	Salbutamol (50 mg/ kg)	-
7) *WS* Gemmo-curative	Normal diet	Salbutamol (50 mg/ kg)	Salbutamol (50 mg/ kg)	Gemmo-extract of *WS* (50 mg/ kg)
8) *WS* Native-curative	Normal diet	Salbutamol (50 mg/ kg)	Salbutamol (50mg/ kg)	Native-extract of *WS* (50 mg/ kg)

Quantitative estimation of antioxidant enzymes: Catalase level in the samples was estimated by the method as describes by Aebi (1974). Peroxidase (POD) activity was measured using the method of Paglia *et al.,* (1967) SOD activity was assayed by using the photochemical NBT method as described by Mishra *et al.,* (1967; Figs. 1-3).

Gross pathological examination: At the end of the experiment, gross pathology of experimental animals was done under the supervision of a Veterinary Doctor. Apparent changes in the weight and structure of the heart, kidneys, liver, stomach and lungs were noted.

Statistical analysis: Descriptive statistics mean and standard deviation were calculated for all the variables of each group. ANOVA was applied for statistical analysis and p value at <0.05 significance level had been considered (Steel & Torrie, 1984).

Results and Discussion

Phytochemical analysis of *W. somnifera* (Asgandh): The results obtained after phytochemical analysis of *W. somnifera* (WS) are given in Table 2.

Cardioprotective effect on *Salbutamol* induced tachycardia: Oral administration of *Salbutamol* (50 mg/ kg) significantly increased ($p<0.05$) the heart beat in gemmo-curative (114 beats/ 30 s) and native-curative (113 beats/ 30 s) groups as compared to normal (95 beats/ 30 s). Heartbeat of rabbits was checked regularly with an interval of 24 h before and after the administration of *Salbutamol*. Post-treatment of ischemic rabbits with gemmo and native extracts of *W. somnifera* (50 mg/ kg)for five days significantly reduced ($p<0.05$) heart beat both in Gemmo-curative (90 beats /30 s) and Native-curative groups (98 beats/ 30 s) as compared to ischemic group (112 beats/ 30 s). Gemmo-extract showed more significant results (90 beats/ 30 s) than native-extract (98 beats/ 30 s).

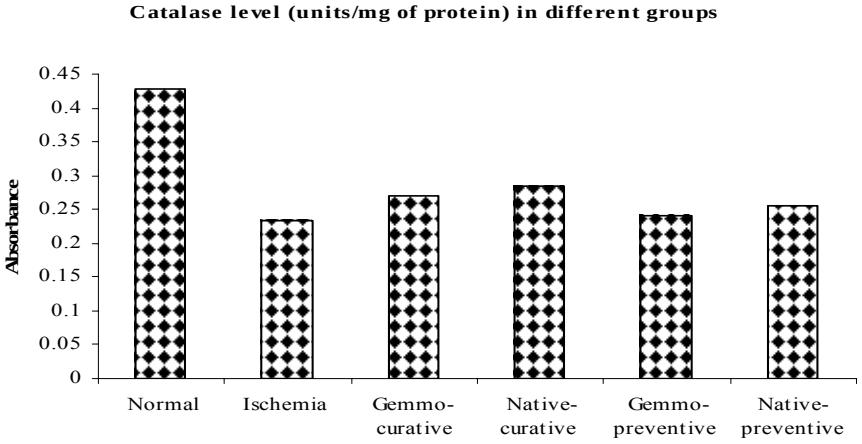

Fig. 1. Catalase level (units/ mg of protein) in different groups.

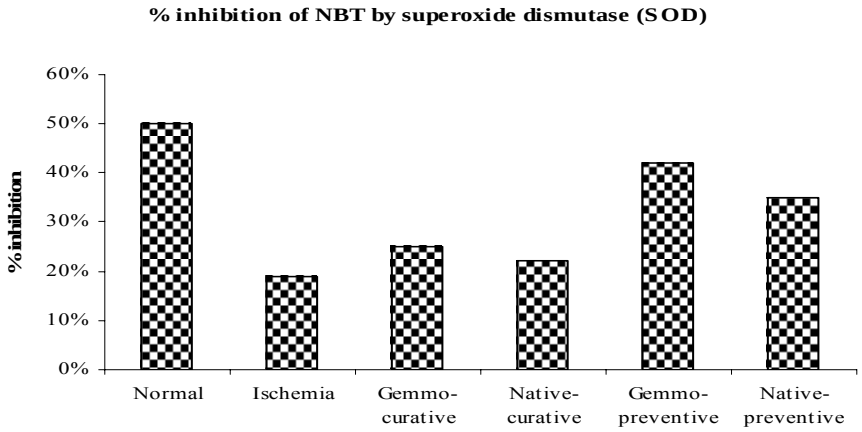

Fig. 2. Percentage inhibition of NBT by superoxide dismutase (SOD).

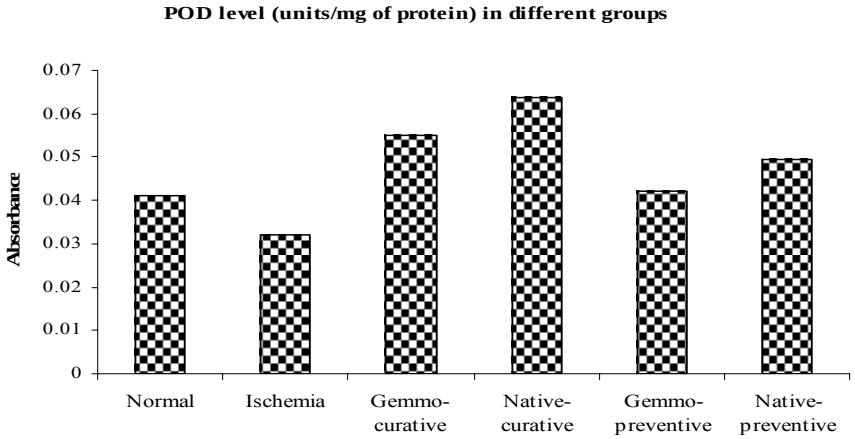

Fig. 3. POD level (units/ mg of protein) in different groups.

Table 2. Phytochemical analysis of *W. somnifera* (WS).

S. No.	Phytconstituents	Results	% Age in native *WS*	% Age in Gemmo *WS*
1.	Alkaloids	+	1.35	2
2.	Flavonoids	+	11.9	10
3.	Glycosides	+	4.3	7.32
4.	Saponins	+	1.88	2.35
5.	Tannic Acid	+	14.75	16
6.	Steroids	+	3.1	5.7

Table 3. Tachycardia values (per 30 s) of different groups.

Days	Normal	Ischemia	Gemmo-curative	Native-curative	Gemmo-preventive	Native-preventive
1	94	90	85	95	93	97
2	93	96	92	101	99	103
3	94	100	106	103	97	110
4	95	118	114	113	101	116
5	95	120	111	112	99	112
6	94	121	105	118	95	107
7	95	119	99	105	96	101
8	93	115	93	99	94	98
9	95	112	90	98	94	95

Table 4. Preventive effect of *W. somnifera* on different enzyme levels (U/L).

Enzymes	Normal	Ischemia	Gemmo Baseline	Native Baseline	Gemmo-preventive	Native-preventive
CK-MB	123.67 ±12.503	306.00 ±12.767	112.23 ±19.183	120.00 ±12.051	186.00 ±27.875	186.33 ±27.220
LDH	238.33 ±7.638	298.33 ±29.297	225.00 ±15.235	242.00 ±13.045	249.67 ±47.385	273.33 ±10.408
SGOT	16.33 ±2.082	30.33 ±4.509	15.20 ±7.41	18.22 ±1.089	19.67 ±1.528	21.33 ±6.110
SGPT	17.67 ±5.686	39.67 ±11.060	14.00 ±3.497	16.23 ±8.25	20.67 ±5.859	±21.67 7.234

Similarly in preventive therapy, pre-treatment of ischemic rabbits with gemmo and native extracts of *W. somnifera* (50 mg/ kg) for 20 days significantly restored ($p<0.05$) the heart beat to normal, both in Gemmo-preventive (94 beats /30 s) and Native-preventive groups (95 beats/ 30 s) as compared to ischemic group (112 beats/ 30 s). Both the Gemmo and native-extract showed similar cardio-preventive effects. Table 3 shows the Tachycardia values (per 30 s) of different groups.

Preventive effect of *W. somnifera* (Table 4): Rabbits were pre-treated with native and gemmo-extract of *W. somnifera* for 20 days and then treated with *Salbutamol* (50 mg/ kg) once a day for two days to induce myocardial injury.

Base-line content of the Gemmo-extract of *W. somnifera* (50 mg/ kg) was found to be CK-MB (112.23 ±19.18), LDH (225±15.235), SGOT (15.20±7.41) and SGPT (14±3.497). Similarly, Base-line content of the Native-extract of *W. somnifera* (50 mg/ kg) was found to be CK-MB (120±12.051), LDH (242±13.045), SGOT (18.22±1.089) and SGPT (16.23±8.25). Administration of *Salbutamol* (50 mg/ kg) significantly increased ($p<0.05$) the serum level of CK-MB (306±12.767) LDH (298.33±29.297), SGOT (30.33±4.509) and SGPT (39.67±11.060) in ischemia group as compared to normal, having CK-MB (123.67±12.503) LDH (238.33±7.638), SGOT (16.33±2.082) and SGPT (17.67±5.686) respectively. However, pre-treatment with gemmo-extract of *W. somnifera* (50 mg/ kg) significantly restored ($p<0.05$) serum enzyme levels to normal in gemmo-preventive group; CK-MB (186±27.875), LDH (249.67±47.385), SGOT (19.67±1.528) and SGPT (20.67±5.859) as compared to ischemia group, having serum level of CK-MB (306±12.767) LDH (298.33±29.297), SGOT (30.33±4.509) and SGPT (39.67±11.060) respectively.

Similarly in native-preventive group, pre-treatment with the native extract of *W. somnifera* significantly restored the serum enzyme levels to normal; CK-MB (186.33±27.22) LDH (273.33±10.408), SGOT (21.33±6.110) and SGPT (21.67±7.234) as compared to ischemia group, having serum enzyme level of CK-MB (306±12.767) LDH (298.33±29.297), SGOT (30.33±4.509) and SGPT (39.67±11.060) respectively.

Both the Gemmo and Native extract of *W. somnifera* showed similar cardio-preventive effects and significantly restored the enzyme levels in gemmo-preventive and native-preventive groups to normal.

Curative effect of *W. somnifera* (Table 5): Ischemia was induced in rabbits by oral administration of *Salbutamol* (50 mg/ kg) for two consecutive days at an interval of 24 h. *Salbutamol* significantly increased ($p<0.05$) the serum levels of CK-MB, LDH, SGOT and SGPT in curative groups as compared to normal. However, post-treatment of ischemic rabbits with gemmo and native extracts of *W. somnifera* (50 mg/ kg) significantly reduced ($p<0.05$) serum levels of CK-MB, LDH, SGOT and SGPT in curative groups as compared to ischemia group in a five-day trial. Table 5 shows the CK-MB, LDH, SGOT and SGPT levels in U/L of different groups. Results exhibited that Gemmo extract showed more activity than Native extract.

Administration of *Salbutamol* (50 mg/kg) for two consecutive days significantly ($p<0.05$) increased serum level of CK-MB in gemmo-curative group (298.67±26.27) and native-curative group (308.00±16.283) as compared to normal group (123.67±12.503). Post-treatment of ischemic rabbits with gemmo and native extracts of *W. somnifera* (50 mg/ kg) for five days significantly ($p<0.05$) reduced serum levels of CK-MB both in Gemmo-curative (148.00±11.790) and Native-curative groups (207.33±45.081) as compared to ischemic group (275.67±10.066). Gemmo-extract showed more significant results (148.00±11.790) than native-extract (207.33±45.081).

Subsequent to the administration of *Salbutamol* (50 mg/ kg) serum level of LDH increased significantly ($p<0.05$) in gemmo-curative group (306.67±08.505) and native-curative group (310.00±9.539) as compared to normal group (238.33±7.638). However, post-treatment of ischemic rabbits with *W. somnifera* for five days significantly ($p<0.05$) reduced serum levels of LDH both in Gemmo-curative (254.67±26.577) and Native-curative groups (260.33±25.502) as compared to ischemic group (284±17.001). Gemmo-extract showed more significant results (254.67±26.577) than native-extract (260.33±25.502).

Table 5. Curative effect of *W. somnifera* on different enzyme levels (U/L).

Enzyme	Day	Normal	Ischemia	Gemmo-curative	Native-curative
CK-MB	1	123.67±12.503	315.00±22.913	298.67±26.270	308.00±16.283
	2	124.00±07.937	315.00±11.136	279.67±30.280	300.00 ± 6.083
	3	121.33±04.040	298.64±20.740	253.67±41.016	290.67±43.240
	4	119.01±10.149	286.00±10.149	192.33±12.220	254.33±29.143
	5	112.33±11.015	275.67±10.066	148.00 ±11.790	207.33 ±45.081
LDH	1	238.33±07.638	313.33±16.073	306.67±08.505	310.00±09.539
	2	232.00±09.165	310.00±09.539	301.00±04.000	294.33±19.009
	3	230.33±16.500	298.33±29.297	280.00±03.606	284.00±17.000
	4	233.33±22.120	272.33±42.063	279.33±15.011	270.00±30.000
	5	232.00±05.292	284.00±17.001	254.67±26.577	260.33±25.502
SGOT	1	16.67±01.52	30.33±04.50	26.67±08.96	32.33±12.05
	2	18.00±01.00	29.67±06.80	21.33±11.01	25.00±05.29
	3	15.33±04.16	25.01±04.35	21.67±09.50	24.67±08.38
	4	15.00±01.00	26.67±01.52	21.33±06.80	21.33±08.14
	5	17.00±3.606	23.67±01.52	18.33±05.68	20.33±06.50
SGPT	1	17.67±05.686	42.33±11.060	39.00±25.530	37.67±04.509
	2	15.67±06.028	39.33±05.508	32.00±10.817	34.67±06.110
	3	15.67±02.082	36.33±07.371	26.33±09.074	31.33±06.506
	4	16.00±01.000	34.00±06.557	26.00±10.536	31.33±04.041
	5	16.33 ± 2.082	29.33±02.082	25.33±09.866	29.00±01.000

Oral administration of *Salbutamol* (50 mg/ kg) for two consecutive days significantly ($p<0.05$) increased serum levels of SGOT in gemmo-curative group (26.67±8.963) and native-curative group (32.33±12.055) as compared to normal group (16.67±1.52). However, post-treatment of ischemic rabbits with *W. somnifera* for five days significantly reduced ($p<0.05$) serum levels of SGOT both in Gemmo-curative (18.33±5.686) and Native-curative groups (20.33±6.506) as compared to ischemic group (23.67±1.52). Gemmo-extract showed a little more significant result (18.33±5.686) than native-extract (20.33±6.506).

Administration of *Salbutamol* (50 mg/ kg) for two consecutive days significantly increased serum level of SGPT in gemmo-curative group (39 ± 25.534) and native-curative group (37.67 ± 4.509) as compared to normal group (17.67 ± 5.686). However, post-treatment of ischemic rabbits with *W. somnifera* for five days did not significantly reduce ($p<0.05$) serum levels of SGPT both in Gemmo-curative (25.33 ± 9.866) and Native-curative groups (29 ± 1) as compared to ischemic group (29.33 ± 2.082). Gemmo-extract showed a little more significant result (25.33 ± 9.866) than native-extract (29 ± 1).

Effect of *W. somnifera* on antioxidant enzymes (Figs. 1-3): Antioxidants are substances that protect other chemicals of the body by damaging oxidation reactions. Antioxidant enzymes include superoxide dismutase, catalase and peroxidase.

Salbutamol-induced ischemic rabbits showed a significant ($p<0.05$) decrease in catalase level (0.233 unit/ mg of protein) as compared to normal (0.428 unit/ mg of protein). Post-treatment of ischemic rabbits in gemmo-curative and native-curative groups showed a significant ($p<0.05$) increase in catalase level (0.271 unit/ mg of protein) and (0.285 unit/ mg of protein) as compared to ischemia group (0.330 unit/ mg of protein). Also, Pre-treatment of ischemic rabbits in gemmo-preventive and native-preventive groups showed a significant ($p<0.05$) decrease in catalase level (0.241 unit/

mg of protein) and (0.256 unit/ mg of protein) as compared to ischemia group (0.330 unit/ mg of protein). Gemmo-extact showed more significant results in both cases (Fig.1). Oral administration of *Salbutamol* showed a significant ($p<0.05$) lower % inhibition (after 15 min.) in ischemic rabbits (19 %) as compared to normal group (50 %). Post-treatment of ischemic rabbits in gemmo-curative and native-curative groups showed a 25% and 22 % inhibition respectively, close to ischemia group (19 %). However, Pre-treatment of ischemic rabbits in gemmo-preventive and native- preventive groups showed a significantly ($p<0.05$) higher 42 % and 35 % inhibition respectively as compared to ischemia group (19 % inhibition).

Salbutamol-induced ischemic rabbits showed a significant ($p<0.05$) decrease in POD level (0.032 unit/mg of protein) as compared to normal (0.041 unit/mg of protein). Post-treatment of ischemic rabbits in gemmo-curative and native-curative groups showed a significant ($p<0.05$) increase in POD level (0.0550 unit/ mg of protein) and (0.0636 unit/mg of protein) as compared to ischemia group (0.032 unit/mg of protein). Also, Pre-treatment of ischemic rabbits in gemmo-preventive and native- preventive groups showed a significant ($p<0.05$) increase in POD level (0.0423 unit/ mg of protein) and (0.495 unit/ mg of protein) as compared to ischemia group (0.032 unit/ mg of protein). Gemmo-extract showed more significant results in both cases (Fig. 3).

Gross pathology results (Tables 6 & 7): Significant changes occurred in different organs of rabbits due to the administration of *Salbutamol*. Heart was normal in all groups except *Salbutamol*-induced ischemia group and native-curative group. Liver was discoloured in all groups. There was no significant change observed in the kidneys. Stomach was damaged in ischemia and curative groups but it was preserved in preventive and normal group. Lungs were affected more seriously having pale red colour in native-preventive and congested in native-curative group. Gemmo-extract showed more significant results in curative therapy.

Discussion

Therapeutic properties of food and medicinal plants arise from the characteristic bioactive phytochemicals (mainly secondary metabolites) synthesized and amassed by them (Sangwan *et al.,* 2004; Walker, 1996). Biologically active plant chemicals other than traditional nutrients that have a beneficial effect on human health have been termed "phytochemicals" (Hasler, 1998).

Our plant showed maximum flavonoids and tannins contents which strongly recommend the antioxidant and cardioprotective nature of *W. somnifera*. (Rastogi & Mehrotra, 1991; Ju, 2005; Lie & Chiou, 1986). Reduction of heart disease *via* dietary intake of phytochemicals has been examined (Fitzpatrick *et al*., 1993; Hertog *et al.,* 1995; Augusti, 1996). The β-adrenoceptor agonists (β-agonists) have been used to relieve bronchoconstriction for at least 5000 years. β-agonists are based on adrenaline and early forms, such as isoprenaline, lacked bronchial selectivity and had unpleasant side effects. Modern β-agonists are more selective for the β2-adrenoceptors (β2-receptors) located in bronchial smooth muscle and have less cardiotoxicity (Sears & Lotvall, 2005; Walker *et al.,* 1985). β2-Adrenoceptor agonists used in the relief of bronchospasm have long been known to produce circulatory side-effects. *Salbutamol* or *albuterol* is a short-acting β2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease. Possible side-effects of *Salbutamol* include tachycardia which may lead to myocardial infarction (Provost *et al.,* 1997; Susan, 1994; Cook *et al.,* 1994; Magnusson and Hansson, 1973).

Table 6. Gross pathological studies of different organs of rabbits.

Organs	Normal	Ischemia	Gemmo-curative	Native-curative	Gemmo-preventive	Native-preventive
Heart	Normal	Hard (damage)	Normal	Hard	Normal	Normal
Liver	Normal	Normal/ pale yellow	Pale yellow	Discoloured	Normal but discoloured	Normal but discoloured
Kidney	Normal	Normal	Normal	Normal	Normal	Normal
Stomach	Normal	Damage	Damage	Damage	Normal	Normal
Lungs	Pale red	Congested	Normal	Congested	Normal	Pale red

Table 7. Weights of different organs of rabbits of different groups.

Organs	Normal	Ischemia	Gemmo-curative	Native-curative	Gemmo-preventive	Native-preventive
Heart	2.13	2.63	2.22	2.81	3.54	3.02
Liver	26.17	29	28.49	27	29.1	28.65
Kidney	7.85	7.2	6.19	6.85	7.14	7.30
Stomach	4.75	5.04	5.8	4.91	5.2	5.01
Lungs	6.4	5.8	6.48	6.02	5.65	5.9

As described above a decrease in the serum levels of CK-MB, LDH, SGOT and SGPT was observed in *Salbutamol* treated groups. Pre and post-treatment with native and gemmotherapeutically treated *W. somnifera* (50 mg/ kg) restore the level of these cardiac markers to normal. Increased activities of CPK, transaminase and Lactate dehydrogenase in serum, 'the diagnostic markers', were due to the leakage of these enzymes as a result of necrosis induced by *Salbutamol* (β-adrenergic stimulant) in rats (Bergstrom & Sawa, 1973). The base-line content of these enzymes were found to be normal which reveals that *W. somnifera* at a dose 50mg/ kg did not induce any cardiotoxic effects. This is according to the previous report presented by Mohanty *et al.,* (2004) that *W. somnifera* at 50 mg/ kg dose produced maximum cardioprotective effect.

Gemmo extract showed more cardio-protective potential than native extract in curative therapy but similar results in preventive therapy. More significant results of the Gemmo-extract than Native-extract is probably due to the fact that the embryonic part of the plant is particularly effective for drainage and detoxifying actions on the human body. Synthetic pharmaceutical agents, most herbs, and homeopathic remedies that are prepared from the whole plant (usually flowering) do not have many of the key elements (growth factors, phytohormones, auxins, and gibberellins) present during the growth stage of plants. This is because the gemmae contain many active principles that start to disappear after a plant reaches a certain point in its development (Daniel & Towle, 2002)

The exact mechanism of this cardioprotective activity is not known but this could be due to the free radical scavenging property of the extract in the presence of antioxidant phytochemicals such as flavonoids, alkaloids, sterols, tannins and phlobatannins and flavonoid glycosides (Rastogi & Mehrotra, 1991; Ju, 2005)

Previous studies also show similar results. A.V.Circulo, a polyherbal preparation, was evaluated in isoproterenol induced myocardial damage in rats. Chronic prophylactic treatment with A.V.Circulo prevented an increase in serum lysosomal enzyme activity of creatinine phosphokinase, lactate dehydrogenase, and serum glutamate oxaloacetic transaminase in the blood due to isoproterenol administration. (Chauhan *et al.,* 2005)

As described above, administration of *Salbutamol* showed a marked decrease in antioxidant enzyme contents in *Salbutamol*-treated groups. However, *W. somnifera* at a

dose 50 mg/ kg increased and restore the antioxidant enzymes content in curative and preventive treatments respectively. Preventive therapy of gemmo-extract proved to be more powerful than curative therapy of gemmo and native extracts. The mechanism of such protection of chronic oral administration of *W. somnifera* may be due to myocardial adaptation, oxidative stress is mediated through augmentation of cellular antioxidants such as peroxidase, SOD, catalase (Das *et al.,* 1995; Gauthaman *et al.,* 2006) Recent studies show that various plants and plant extracts can also stimulate the synthesis of cellular antioxidants (Pathania *et al.,* 1998; Gauthaman *et al.,* 2001; Banerjee *et al.,* 2002; Fig. 2). This is in accordance to the previous report that medicinal plants used in the therapy of cardiovascular disease exert their beneficial effects *via* antioxidant activity. There is a growing body of evidence suggesting that antioxidants contribute to cardioprotection (Munasinghe *et al.,* 2001).

Gross pathology refers to macroscopic manifestations of disease in organs, tissues, and body cavities. The term is commonly used by anatomical pathologists to refer to diagnostically useful findings made during the gross examination portion of surgical specimen processing or an autopsy. The gross pathological examination of different organs of animals recommended the cardioprotective potential of *W. somnifera*. Gross pathological confirmation of cardiotoxic effect produced by *Salbutamol* (50 mg/ kg), in the present investigation has established the suitability of this model for studying the cardioprotective effect of *W. somnifera* (Mohanty *et al.,* 2004) Gross pathology results recommend the efficacy of preventive therapy as compared to curative therapy.

The major active constituents of *W. somnifera* through which it exhibits medicinal properties, are based upon the actions of certain steroidal alkaloids and lactones as a class of constituents called withanolides. The root contains the steroidal lactone (withaferin A) and related withanolides, along with various alkaloids. It is reported that Sitoindosides VII, VIII, IX and X are likely adaptogenically active substances present in *Withania somnifera*. The exact mechanism of such myocardial adaptation is not known. However, it is proposed that it works through the induction of a number of antioxidant enzymes (Archana & Namasivayam, 1999).

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